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High glucose concentration stimulates intracellular renin activity and angiotensin II generation in rat mesangial cells

D. B. Vidotti, D. E. Casarini, P. C. Cristovam, C. A. Leite, N. Schor, and M. A. Boim

Renal Division, Federal University of São Paulo, 04023-900 São Paulo, Brazil

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Vidotti, D. B., D. E. Casarini, P. C. Cristovam, C. A. Leite, N. Schor, and M. A. Boim. High glucose concentration stimulates intracellular renin activity and angiotensin II generation in rat mesangial cells. *Am J Physiol Renal Physiol* 286: F1039–F1045, 2004. First published January 13, 2004; 10.1152/ajprenal.00371.2003.—Increased intrarenal renin-angiotensin system activity contributes to diabetic nephropathy. ANG II generation in mesangial cells (MC) is increased by high-glucose (HG) exposure. This study assessed the mechanisms involved in the glucose-induced ANG II generation in rat MC. Under basal conditions, MC mainly secreted prorenin. HG decreased prorenin secretion and induced a striking 30-fold increase in intracellular renin activity. After 72 h of HG exposure, only the mRNA levels for angiotensinogen and angiotensin-converting enzyme (ACE) were significantly elevated. However, after shorter periods of 24 h of HG stimulation the mRNA levels of the enzymes prorenin and cathepsin B, besides that for ACE, were significantly increased. The results suggest that the HG-induced increase in ANG II generation in MC results from an increase in intracellular renin activity mediated by at least three factors: a time-dependent stimulation of (pro)renin gene transcription, a reduction in prorenin enzyme secretion, and an increased rate of conversion of prorenin to active renin, probably mediated by cathepsin B. The increase in angiotensinogen mRNA in parallel to increased renin activity indicates that HG also increased the availability of the renin substrate. The consistent upregulation of ACE mRNA suggests that, besides renin, ACE is directly involved in the increased mesangial ANG II generation induced by HG.

hyperglycemia; diabetic nephropathy; glomerular sclerosis; renin-angiotensin system

DIABETIC NEPHROPATHY IS CHARACTERIZED by excessive production of mesangial matrix that contributes to glomerular sclerosis, leading to proteinuria and renal failure. Among the potential mediators of mesangial matrix expansion in diabetic nephropathy, the peptide ANG II is particularly outstanding, and many lines of evidence suggest activation of the intrarenal renin-angiotensin system (RAS) in diabetes (21). The RAS has been extensively studied in diabetes, and strong evidence indicates that the inhibition of angiotensin-converting enzyme (ACE) and/or ANG II receptors delays the progression of diabetic nephropathy (3, 37). Despite these benefits, the renoprotective effects of RAS inhibition cannot be fully explained only by the hemodynamic effects of the system causing a reduction in blood pressure. Moreover, many patients with diabetic nephropathy paradoxically present low plasma renin activity (12, 32). Thus renin suppression in this condition supports the hypothesis that, rather than systemic synthesis, increased local synthesis of ANG II in the kidney may contribute to the pathogenesis of diabetic nephropathy, although the specific site of intrarenal ANG II in this pathological condition is not completely known.

Renal tubular epithelial cells constitute a potential site of intrarenal ANG II synthesis (25, 35). In addition, it has been demonstrated that mesangial cells express mRNA for renin, angiotensinogen, and ACE (4, 5). Renin is a rate-limiting enzyme in the synthesis of ANG II (34), and we have recently demonstrated that mesangial cells in culture are able to synthesize, store, and secrete both forms of the enzyme, i.e., active renin and inactive prorenin (4).

Hyperglycemia has been recognized to be the pathogenic factor of the long-term complications of diabetes mellitus. Thus in the present study we examined whether a high glucose concentration in the culture medium is able to modify renin synthesis, the secretion of the renin/prorenin ratio, and the intracellular renin activity in mesangial cells in culture. Also, we examined whether this "hyperglycemic" condition alters the levels of mRNA expression of RAS components, including angiotensinogen, prorenin, ACE, types I and II (AT_1 and AT_2) angiotensin receptors, and cathepsin B, a potential enzyme involved in renin activation. Finally, we also determined whether glucose-inducing modifications in renin activity and in the expression of RAS components result in an increase in local ANG II generation.

MATERIALS AND METHODS

Primary culture of rat mesangial cells. Mesangial cells were cultured by standard techniques using glomerular isolation by differential sieving (17). Briefly, glomerular mesangial cells were cultured from kidneys freshly removed from normal adult male Wistar rats. The kidney cortex was macrodissected, fragmented, forced through a graded series of stainless steel sieves (60, 100, and 200 mesh), and rinsed with RPMI 1640 culture medium. The glomeruli were then collected from the surface of the third sieve and forced through a 25 × 7-gauge needle for full decapsulation. Mesangial cells were obtained from collagenase-treated, isolated glomeruli to remove the epithelial cell component. Glomeruli were plated at a density of ~300 glomeruli/cm onto RPMI 1640 containing 10 mM D-glucose and supplemented with 20% fetal calf serum, 50 U/ml penicillin, 2.6 g acid HEPES, and 2 mM glutamine. Culture flasks were kept in a 95% air-5% CO_2 humidified environment at 37°C. The culture medium was replaced every 36 h. After 3 wk, cells were harvested with trypsin and the subcultures were grown in the same culture medium. Cells were used between the third and fifth subculture and characterized by classic methods using the following criteria: morphological appearance of stellate cells, immunofluorescence staining of the extracellular matrix for type IV collagen and fibronectin, negative immunofluorescence staining for human factor VIII antigens (glomerular endothelial

Address for reprint requests and other correspondence: M. A. Boim, Renal Div., UNIFESP, Rua Botucatu, 740, 04023-900 São Paulo, Brazil (E-mail: mirian@nefro.epm.br).

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cells) and cytokeratin (parietal epithelial cells), and positive immunofluorescence staining for actin and myosin. In particular, the presence of contaminating juxtaglomerular cells was excluded on the basis of their characteristic phenotype including their spherical shape and the presence of a high density of granules.

Experimental protocol. At subconfluence, mesangial cells were divided into three groups as follows: control, where cells were kept in RPMI 1640 medium supplemented with FBS, containing normal glucose concentration of 10 mM D-glucose (NG group); high glucose, where cells were cultured in RPMI 1640 supplemented with FBS containing 30 mM D-glucose (HG group); and mannitol, where cells were submitted to the same procedure as used for the cell culture in the presence of glucose, but 30 mM mannitol was added instead of glucose and served as osmolarity control (M group). Cells were exposed to 30 mM D-glucose or mannitol for a total of 72 h (16). After 48 h, cells were rinsed twice with PBS and the culture medium was replaced with a medium containing the respective normal- or high-glucose and mannitol concentrations, but no FBS, to keep the cells in the G_0 phase of the cell cycle and without any interference from serum proteins. The culture medium was then collected over the last 24 h and stored at −70°C until use. Cells were rinsed with PBS, lysed with 1 mM Tris·HCl buffer, pH 7.5, and stored at −70°C until use. An additional group of cells were exposed to high glucose concentration for a shorter period of 24 h.

Determination of active and inactive renin (prorenin) content and renin activity. The renin content of the active and inactive forms of renin was estimated by measuring the amount of ANG I present in the cell lysate and culture medium by HPLC, as described below. Total renin content included active renin and the inactive prorenin forms. Prorenin was activated by adding 10 μl of trypsin (50 μg/ml) for 16–18 h at 37°C to both the cell lysate and culture medium. Active renin was evaluated in the absence of trypsin. To prevent further cleavage of prorenin, angiotensinogen, and ANG I, a pool of enzymatic inhibitors was added to the cell homogenate and culture medium: 50 mM EDTA, 1 mM OPhe, 32 mM PMFS, and 200 mM DTT. These inhibitors act efficiently on a broad range of metallo-, serine, and cysteine proteases, respectively. Renin activity was estimated by ANG I generation when the cell lysate and culture medium (in the presence and absence of trypsin) were incubated with 10 μl of 1 mg/ml synthetic tetradecapeptide substrate (Sigma, St. Louis, MO) for 4 h at 37°C, as previously described (24). The reaction was stopped by adding 10 μl of 50% H_3PO_4. One hundred microliters of each sample were filtered and injected into the HPLC system. The released ANG I peptide was quantified by reverse-phase HPLC using an aquapore ODS 300 column equilibrated with 0.1% phosphoric acid containing 5% acetonitrile (vol/vol). ANG I was separated by isocratic elution for 5 min, followed by a 20-min linear gradient of 5–35% acetonitrile in 0.1% phosphoric acid (vol/vol) at 1.5 ml/min. The chromatographic profile of each sample was compared with that obtained for standard samples containing angiotensinogen and ANG I at an absorbance of 214 nm. Peptide fragments were identified by elution position and quantified by integration area using repeated injections of standard peptide solution to correct for small differences in retention time (<6%) and peak height (<5%). The results were corrected by the amount of intracellular protein determined by the method of Lowry. All reagents used for renin analysis were purchased from Sigma.

Identification of RAS component mRNA expression by RT-PCR. The expression of mRNAs of the RAS components in mesangial cells was determined by RT-PCR. Total RNA was purified from cells by the phenol and guanidine isothiocyanate-cesium chloride method using an appropriate kit (TRIzol, Life Tecnologies). The RNA pellet was resuspended in RNase-free water. Total RNA concentration was estimated with a spectrophotometer (Gene Quant RNA/DNA calculator, Amersham Pharmacia Biotech, Uppsala, Sweden). Two micrograms of total RNA were reverse transcribed into cDNA by the addition of a mix containing 0.5 mg/ml oligo dT, 10 mM DTT, 0.5 mM dNTPs (Pharmacia Biotech), and 200 U of RT enzyme (SuperScript RT, GIBCO BRL). The mixture was incubated at 37°C for 1 h and then at 95°C for 5 min. PCR was performed in a thermal cycler (model PTC-100, MJ Research, Watertown, MA) using 2 μl of reverse-transcribed cDNA in a total volume of 20 μl containing 1.0–2.5 mM MgCl (optimized for each primer pair), 0.5 mM of each primer, 0.5 mM dNTP mix, and 0.5 U *Taq* DNA polymerase (Pharmacia Biotech). Primer sequences for amplification of angiotensinogen, prorenin, ACE, and AT_1 and AT_2 receptors were designed using appropriate software (PCR-Designer, Research Genetics) based on each cDNA sequence obtained from GenBank. Primer sequences and the amplification conditions for each primer are shown in Table 1. Negative controls were included in each PCR set where cDNA was replaced with water as a control for contamination from exogenous sources. In addition, the RT enzyme was omitted in some samples as a negative control for amplification of genomic DNA. Positive controls included kidney cortex cDNA and the efficiency of the RT reaction was monitored by the amplification of the constitutively expressed gene for β-actin. PCR products were electrophoresed on 1% agarose gels and visualized by ethidium bromide staining under UV light.

Expression levels of RAS components by quantitative real-time PCR analysis. Because the presence of RAS components was confirmed by RT-PCR, the expression levels of each mRNA were estimated by real-time RT-PCR using a GeneAmp 5700 Sequence Detection System SDS (ABI Prism 7700, Applied Biosystems), which was developed to determine gene expression with high sensitivity and specificity. The cDNA was synthesized from 1 μg of total RNA extracted from each group of cells as described above. Real-time PCR product accumulation was monitored using the intercalating dye SYBR Green I (Molecular Probes), which exhibits a higher fluorescence on the binding of double-strand DNA. Relative gene expression was calculated using conditions at the early stages of PCR, when amplification was logarithmic and thus could be correlated to the initial copy number of gene transcripts. The reactions were cycled 40 times under the conditions previously determined by conventional PCR. Fluorescence for each cycle was quantitatively analyzed by ABI Prism 7700 SDS (Applied Biosystems). At the end of the PCR, the temperature was increased from 60 to 95°C at a rate of 2°C/min, and fluorescence was measured every 15 s to construct the melting curve. A nontemplate control was run with each assay. The relative amount of each mRNA was estimated using a standard curve constructed from serial dilutions of cDNA including 1:1, 1:10, and 1:100. The results of five experiments per group are reported as relative expression normalized with the GAPDH housekeeping gene, used as an endogenous control, and expressed in arbitrary units.

ANG II measurement by ELISA. ANG II levels in the cell lysate were determined by ELISA using the avidin-streptavidin method as previously described (38). Five micrograms of protein samples were

Table 1. *PCR primer sequences and product size*

Molecule	Primer Sequence	Product Size, bp
Angiotensinogen	5′-TCCCTGTCCTGTAATCCCTC-3′ 5′-GGCTGCTGCTCATCATTTAT-3′	398
Prorenin	5′-AAGGTTTCCTCAGCCAAGAT-3′ 5′-AAGTTGCCCTGGTAATGTTG-3′	295
ACE	5′-GCAGAACTTCACTGACCAAAAG-3′ 5′-TCAAAGGAGGGGGACTCATA-3′	228
AT_{1B} receptor	5′-ATGCCAGTGTGTTTCTGCTC-3′ 5′-CCAATGGGGAGTGTTGAGTT-3′	244
AT_2 receptor	5′-GTGTCCAGCATTTACATCTTCA-3′ 5′-CACCAAACAAGGGGAACTAC-3′	275
Cathepsin B	5′-GGGGGAAATCTACAAAAATG-3′ 5′-AAAGACTCCTATCTGCCTCACT-3′	388

ACE, angiotensin-converting enzyme.

added to the wells of a microtiter plate in duplicate. Protein was allowed to adsorb for 1 h at 37°C and then blocked with 200 μl of PBS-Tween 20 containing 0.5% casein for 2 h at 37°C, and the anti-ANG II antibody (1:100 dilution) was added to the respective well and incubated for 1.5 h at 37°C. Plates were washed three times with PBS-Tween 20, and 150 μl of biotinylated anti-mouse IgG (1:500 dilution, Life Science) were added to each well and incubated for 1 h at 37°C. Samples were washed and then incubated with 100 μl of streptavidin-peroxidase (1:2,000 dilution, Amersham Life Science) for 45 min at 37°C. The final wash was followed by development using *o*-phenylenedimine dihydrochloride (Abbott Laboratories) and hydrogen peroxidase reagents for 20 min at room temperature. Absorbance was recorded at 495 nm, and ANG II concentrations were calculated from the standard curve.

Statistical analysis. Results are expressed as means ± SE. Data were analyzed by one-way ANOVA followed by the Tukey or Newman-Keuls test. Nonparametric data were analyzed by the Dunn or Kruskal-Wallis test. $P < 0.05$ was considered significant.

RESULTS

Total renin content, including active renin and prorenin, was measured in the cell lysate and culture medium. Renin content was estimated in the presence of an excess of substrate. In the cell lysate it corresponds to the amount accumulated over 72 h, and the content in the culture medium corresponds to the amount accumulated over the last 24 h of the experimental protocol. The amount of prorenin was calculated from the difference between the content of total and active renin. The content of renin was estimated from renin activity, obtained with an excess of substrate. Figure 1 represents the content of active and inactive renin in both cell lysate and culture medium. High glucose concentration induced an impressive elevation in the intracellular content of active renin as well as in the prorenin content compared with the control groups (NG and M). In contrast, in the culture medium the content of active renin was similar for the three groups, but the amount of prorenin was reduced by HG compared with control cells.

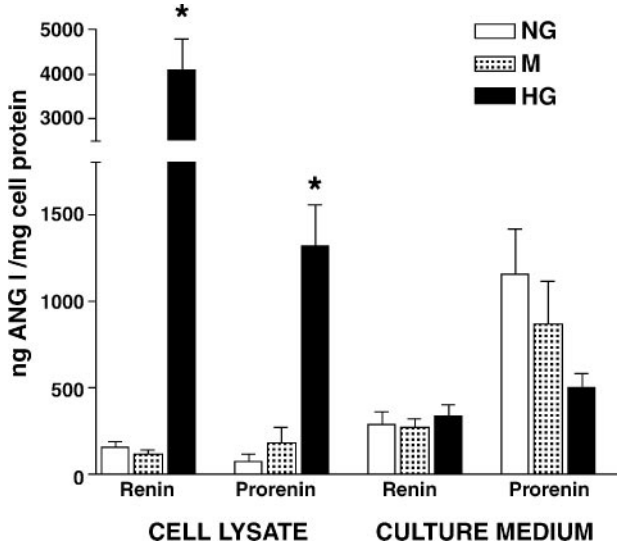

Fig. 1. Renin and prorenin content in the cell lysate and culture medium. The content of renin and prorenin was estimated in the mesangial cell lysate after 72 h of exposure to normal (NG; 10 mM) or high (HG; 30 mM) glucose or mannitol (M; 30 mM). The content obtained in the culture medium refers to the amount of renin forms secreted during the last 24 h. Values were estimated from the amount of ANG I measured by HPLC. Prorenin was activated by adding 10 μl of trypsin (50 μg/ml) for 16–18 h at 37°C. Active renin was evaluated in the absence of trypsin. Values are means ± SE of 6 experiments/group. *$P < 0.05$ vs. control (NG) and the M group.

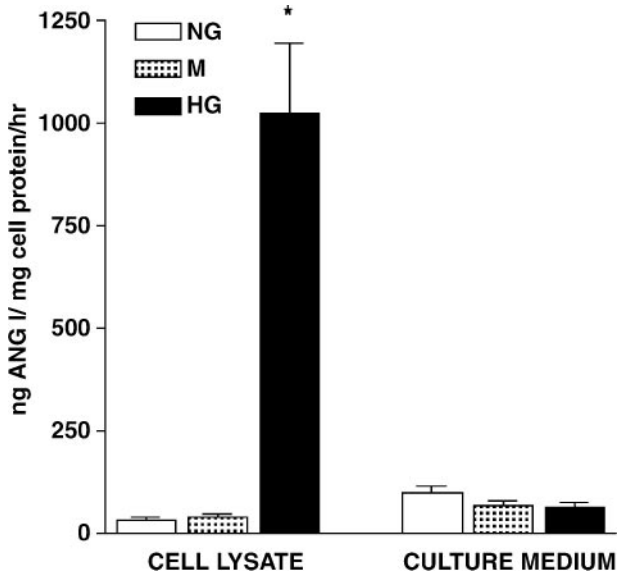

Fig. 2. Renin activity. Renin activity was obtained by incubating the cell lysate and culture medium with the renin substrate for 4 h at 37°C. Values were estimated from the amount of ANG I generated in the absence of trypsin. Values are means ± SE of 6 experiments/group. *$P < 0.05$ vs. NG and M groups.

Figure 2 shows renin activity expressed as nanograms of ANG I per milligram protein per hour in the cell lysate and culture medium. Besides increasing renin content, a high glucose concentration also induced a significant 30-fold increase in the renin activity measured in the cell lysate compared with the control and M groups but did not change renin activity in the culture medium.

The expression of mRNAs for angiotensinogen, cathepsin B, prorenin, ACE, and AT_1 and AT_2 receptors was detected in mesangial cells by RT-PCR (data not shown). After optimization of the PCR conditions for each primer pair, specific for each component, cDNA samples were used to quantify the mRNAs by real-time PCR. Figure 3 shows a representative standard curve and amplification curves for ACE in the three groups. A threshold was determined for each PCR run to match the logarithmic phase of the curve, and the amount of each cDNA was estimated from the standard curve. As can be observed in this representative curve for ACE, the control samples (NG and M) were closer to the most diluted points (1:100 and 1:10), whereas the HG samples coincided with the undiluted point.

Figure 4 shows the relative quantification of mRNAs for all RAS components. Data are expressed in arbitrary units considering GAPDH as the internal control. The mRNA expression levels detected after 72 h of high-glucose exposure were unchanged for all components except angiotensinogen, 1.8-fold higher than in the NG group, and mainly ACE mRNA, which presented a 15-fold increase compared with the NG group. In addition, the mRNA expression levels of the RAS enzymes including prorenin, cathepsin B, and ACE were further estimated after 24 h of high-glucose exposure. Figure 5 shows that after this shorter period the mRNA expression

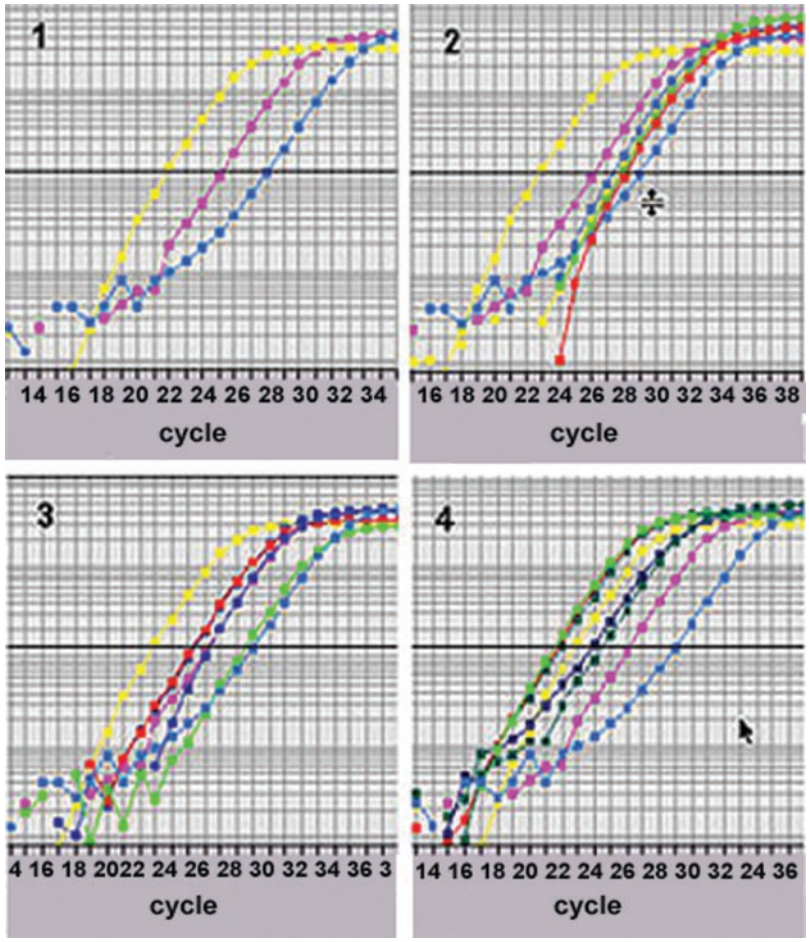

Fig. 3. Representative standard curve and real-time PCR run. The standard curve was constructed from serial dilutions of cDNA using angiotensin-converting enzyme (ACE)-specific primers (*1*) and representative PCR run for ACE in groups NG (*2*), M (*3*), and HG (*4*). See text for details.

levels for the three enzymes were significantly elevated in the HG group compared with the NG group.

ANG II concentration in mesangial cells was determined by ELISA. As shown in Fig. 6, glucose-stimulated cells presented a significant increase in ANG II generation compared with control cells. Mannitol-exposed cells also presented an increase in ANG II, but the mean value obtained did not differ from control cells.

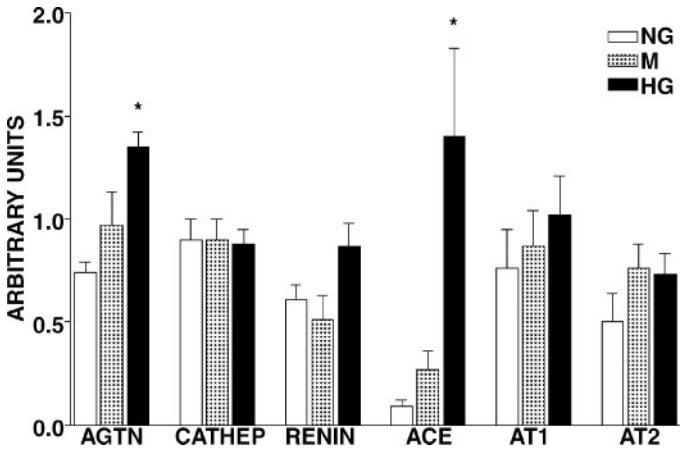

Fig. 4. Quantitative real-time PCR for renin-angiotensin (AGTN) system (RAS) components and cathepsin B (CATHEP). Total RNA was isolated from pooled cells obtained from 4 or 5 culture flasks from each group. The relative amount of mRNAs was estimated by real-time PCR, normalized by GAPDH used as endogenous control, and expressed as arbitrary units. Values are means ± SE of 5 experiments/group after 72 h of stimulation with mannitol or high glucose. *$P < 0.05$ vs. control.

DISCUSSION

Many lines of evidence suggest a role for intrarenally formed ANG II in the pathogenesis of diabetic nephropathy (12, 21, 32). It has been shown that glucose and ANG II are able to increase the synthesis of collagen types I and IV and other matrix proteins in mesangial cells, as well as to decrease the levels of proteases involved in matrix degradation, resulting in the matrix expansion observed in diabetic nephropathy (19, 37). It has been shown that increasing glucose concentrations cause proportional increases in ANG II generation in mesangial cells (38). In the present study, we obtained further evidence that mesangial cells may contribute to the enhancement of intrarenal ANG II observed in diabetic kidneys.

We have shown that mesangial cells are able to synthesize, store, and spontaneously secrete both the active and inactive forms of renin, as previously demonstrated by us in rat and mouse mesangial cells (4) and by Chansel et al. (10) in human mesangial cells. Moreover, it was shown that the secretion rate of active and inactive renin changes in response to the external stimulus induced by high glucose concentration in the culture medium. In control and mannitol-stimulated cells, prorenin was predominant in the culture medium, indicating that under basal conditions most of the renin secreted is in the inactive

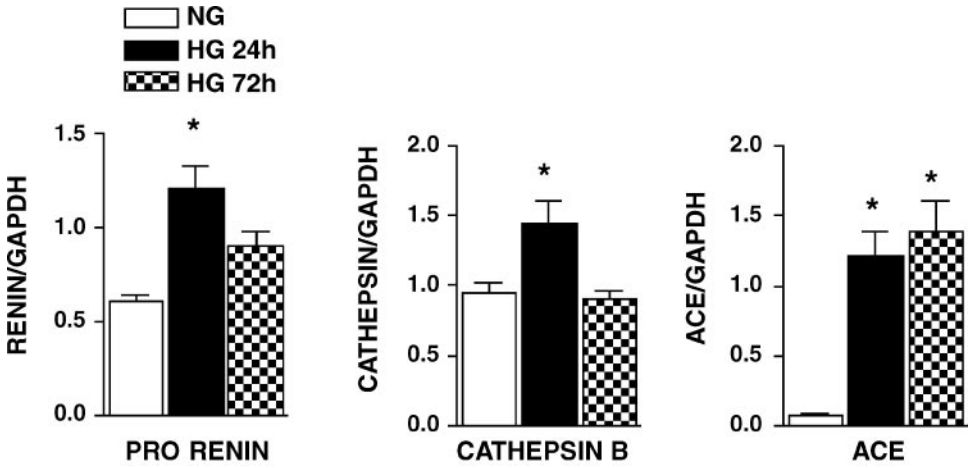

Fig. 5. Quantitative real-time PCR for prorenin, cathepsin B, and ACE. mRNA expression levels for the enzymes were quantified after a shorter period of glucose incubation (24 h) and compared with 72 h of incubation. Quantification was performed by real-time PCR using GAPDH as the endogenous control. Values are means ± SE of 5 experiments/group expressed in arbitrary units. *$P < 0.05$ vs. NG group.

form, as also described for juxtaglomerular cells and other renin-secreting cells (9, 15, 18). The reason mesangial cells secrete large quantities of inactive renin is not known, but the presence of renin and prorenin receptors in the kidney (29) and mesangial cells (28) suggests an exciting possibility of autocrine and paracrine functions for prorenin and renin secreted by mesangial cells.

High glucose induced a reduction in renin content in the culture medium, mainly in prorenin secretion, roughly three times that observed in control cells, resulting in the accumulation of prorenin in the intracellular compartment. It was recently demonstrated that the mesangial renin receptor binds renin and prorenin, and the binding of renin to this receptor, in human mesangial cells, induced a fourfold increase in the efficiency of angiotensinogen conversion to ANG I (29) compared with renin in the soluble phase. Moreover, the renin/prorenin binding to the receptor would not be detected in the culture medium but in the cell lysate fraction. Whether the increased prorenin content in the intracellular compartment observed in the present study was a result of reduced secretion and/or a binding to the receptor deserves further investigation. In addition, intracellular prorenin accumulation was also a result of increased prorenin gene transcription, observed after a short period of high-glucose stimulation. Actually, one of the most important events observed in the present study was an impressive rise in intracellular renin content and activity induced by high-glucose exposure, suggesting that the accumulated prorenin was converted to active renin inside the cell, and thus high glucose also induced an increase in the rate of prorenin-to-renin conversion. This result is in contrast to that recently described by Singh and co-workers (38). They found that renin activity, measured as ANG I generation in the presence of an excess of angiotensinogen, was not altered by high glucose. We have no explanation for this discrepancy, but maybe some differences between the protocols used to measure renin activity would be considered. For instance, the aforementioned study does not mention whether the protease inhibitors were added to the renin assay, as in the present study, and this is particularly important because renin appears to be rapidly inactivated in vitro and probably by proteases (13).

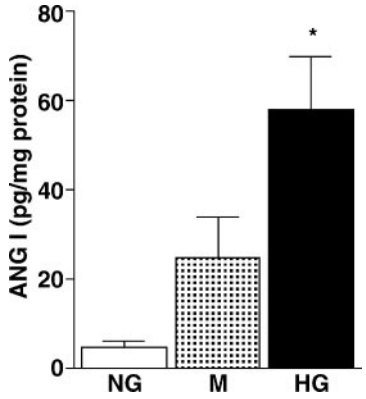

Fig. 6. Intracellular ANG II concentration. ANG II was measured by ELISA in the cell lysate after 72 h of exposure to normal or high glucose or mannitol. Values are means ± SE of 5 experiments. *$P < 0.05$ vs. NG group.

The conversion of prorenin to active renin results from the proteolytic cleavage of 43 amino acids from the pro-segment of prorenin. Although many enzymes have been implicated in this mechanism, cathepsin B has been suggested to be the primary one involved in the activation of prorenin in vivo (27). The presence of cathepsin B-like activity was recently demonstrated in rat mesangial cells, in parallel to its ability to hydrolyze prorenin (1), making this enzyme a potential candidate involved in the conversion of prorenin to active renin in mesangial cells. We found a significant increase in cathepsin B mRNA expression levels after 24 h of high-glucose exposure, possibly indicating that the increase in the conversion of prorenin to active renin induced by high glucose was, at least initially, mediated by cathepsin B. On the other hand, cathepsin B mRNA returned to control levels after the more prolonged period of 72 h of glucose stimulation. Actually, previous reports have shown that the diabetic milieu is mostly associated with a decrease in the degradative enzymes, including cathepsin B, in glomeruli and mesangial cells (22, 23, 39). Taken together, these data raise the possibility that glucose may interfere with cathepsin B expression through a time-dependent pathway, initially stimulating and then suppressing cathepsin B gene transcription.

Similar to cathepsin B mRNA expression, high glucose induced an initial increase in prorenin mRNA, detected after 24 h, with a return to control levels after 72 h of glucose exposure, suggesting that renin synthesis by mesangial cells is probably subjected to the usual negative feedback control

induced by ANG II as classically observed in juxtaglomerular cells (26).

In contrast to time-dependent upregulation of prorenin and cathepsin B gene transcription, the expression levels of ACE mRNA were significantly elevated after both 24 and 72 h of high-glucose stimulation, suggesting that ACE is probably involved in the increased mesangial ANG II generation by glucose. However, whether ACE gene transcription is influenced by high glucose and/or by ANG I concentration deserves additional investigation.

High glucose induced an upregulation of angiotensinogen gene expression detected after 72 h. Singh et al. (38) recently demonstrated that mesangial cells incubated in high-glucose media increased both angiotensinogen mRNA and protein levels. Taken together, these data suggest that, in parallel to increased renin activity, high glucose also increased the availability of the renin substrate.

The results showed that mesangial cells in culture are able to constitutively express mRNA for both AT_1 and AT_2 receptors, as also previously demonstrated (6, 11, 20, 35). In contrast to intracellular components, high glucose did not change mRNA expression levels for AT_1 or AT_2 receptors. Conflicting results have been reported concerning the effect of glucose or diabetes on the expression of ANG II receptors in kidney cells. Downregulation of the AT_1 receptor has been demonstrated in glomeruli and proximal tubules of diabetic rats (7, 30, 40), as well as in mesangial cells chronically incubated in culture medium containing high glucose levels (2). In contrast, Chouinard and co-workers (11) recently demonstrated that high glucose did not modify AT_1 mRNA expression in mesangial cells but increased the expression of AT_2 receptors. On the other hand, the absence of changes in ANG II receptor mRNA observed in the present study does not rule out the possibility of an upregulation of gene transcription during shorter periods of glucose stimulation. Finally, it is important to reflect on the possibility that ANG II may not leave the cells to induce its effects. Previous data from our laboratory (unpublished observations) showed ANG II immunostaining in the nucleus of mesangial cells, which was strongly increased after exposure to high glucose levels. ANG II accumulation inside the nucleus has been found in other tissues, including myocardium, brain, smooth muscle, and adrenal glands (8, 14, 32). The presence of ANG II inside the nucleus may represent an exciting mechanism involved in the well-known effects of ANG II-inducing gene expression, particularly those involved in cell growth, metabolism, and synthesis of extracellular matrix components, manifestations that are typical of diabetic nephropathy.

In summary, the present study showed a significant increase in ANG II generation in mesangial cells exposed to a high glucose concentration. The results suggest that the mechanisms involved in the upregulation of mesangial ANG II primarily include an increase in intracellular renin activity, which resulted from at least three mechanisms, i.e., a time-dependent stimulation of prorenin gene transcription, a reduction in prorenin secretion, and an increase in the rate of prorenin conversion to active renin, probably mediated by cathepsin B. The increased renin activity was paralleled by a rise in angiotensinogen gene expression, indicating availability of the substrate to renin. In contrast to time-dependent upregulation of prorenin and cathepsin B gene transcription, the expression levels of ACE mRNA were significantly elevated after both 24 and 72 h of high-glucose stimulation, indicating that ACE upregulation is probably necessary to increase ANG II synthesis. The mRNA levels for ANG II receptors were unchanged, at least after 72 h of exposure to glucose, suggesting that ANG II production by mesangial cells under glucose stimulation may serve as an intracrine action, thus directly mediating the proliferative and inflammatory effects of ANG II, with consequent cell proliferation, matrix expansion, and inflammation contributing to the glomerular sclerosis observed in diabetic nephropathy.

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REFERENCES

1. **Almeida PC, Oliveira V, Chagas JR, Cezari MH, Juliano MA, and Juliano L.** Hydrolysis by cathepsin B of fluorescent peptides derived from human prorenin. *Hypertension* 35: 1278–1283, 2000.
2. **Amiri F and Garcia R.** Regulation of angiotensin II receptors and PKC isoforms by glucose in rat mesangial cells. *Am J Physiol Renal Physiol* 276: F691–F699, 1999.
3. **Andersen NH and Mogensen CE.** Angiotensin converting enzyme inhibitors and angiotensin II receptor blockers: evidence for and against the combination in the treatment of hypertension and proteinuria. *Curr Hypertens Rep* 4: 394–402, 2002.
4. **Andrade AQ, Casarini DE, Schor N, and Boim MA.** Characterization of renin mRNA expression and enzyme activity in rat and mouse mesangial cells. *Braz J Med Biol Res* 35: 17–24, 2002.
5. **Andrade MC, Quinto BM, Carmona AK, Ribas OS, Boim MA, Schor N, and Casarini DE.** Purification and characterization of angiotensin I-converting enzymes from mesangial cells in culture. *J Hypertens* 16: 2063–2074, 1998.
6. **Ardaillou R, Chansel D, Chatziantoniou C, and Dussaule JC.** Mesangial AT_1 receptors: expression, signaling, and regulation. *J Am Soc Nephrol* 10, *Suppl* 11: S40–S46, 1999.
7. **Ballermann BJ, Skorecki KL, and Brenner BM.** Reduced glomerular angiotensin II receptor density in early untreated diabetes mellitus in the rat. *Am J Physiol Renal Fluid Electrolyte Physiol* 247: F110–F116, 1984.
8. **Booz GW, Conrad KM, Hess AL, Singer HA, and Baker KM.** Angiotensin-II-binding sites on hepatocyte nuclei. *Endocrinology* 130: 3641–3649, 1992.
9. **Carey RM, McGrath E, Pentz ES, Gomez RA, and Barrett PQ.** Biomechanical coupling in renin-releasing cells. *J Clin Invest* 100: 1566–1574, 1997.
10. **Chansel D, Dussaule JC, Ardaillou N, and Ardaillou R.** Identification and regulation of renin in human cultured mesangial cells. *Am J Physiol Renal Fluid Electrolyte Physiol* 252: F32–F38, 1987.
11. **Chouinard RF, Meek RL, Cooney SK, and Tuttle KR.** Effects of amino acids, and glucose on mesangial cell aminopeptidase a and angiotensin receptors. *Kidney Int* 61: 106–109, 2002.
12. **Christlieb AR, Kaldany A, and D'elia JA.** Plasma renin activity an hypertension in diabetes mellitus. *Diabetes* 25: 969–974, 1976.
13. **De Vito E, Guardia DC, and Martinez de Melian ER.** Spontaneous inhibition or inactivation of renin in rat plasma. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 116: 55–60, 1997.
14. **Erdmann B, Fuxe K, and Ganten D.** Subcellular localization of angiotensin II immunoreactivity in the rat cerebellar cortex. *Hypertension* 28: 818–824, 1996.
15. **Hackenthal E, Paul M, Ganten D, and Taugner R.** Morphology, physiology, and molecular biology of renin secretion. *Physiol Rev* 70: 1067–1116, 1990.

16. **Hadad SJ, Michelacci YM, and Schor N.** Proteoglycans and glycosaminoglycans synthesized in vitro by mesangial cells from normal and diabetic rats. *Biochim Biophys Acta* 1290: 18–28, 1996.
17. **Hadad SJ, Souza ERM, Ferreira AT, Oshiro MEM, Boim MA, Razvickas CV, Moura LAR, and Schor N.** FK 506: effects of glomerular hemodynamics and on mesangial cells in culture. *Kidney Int* 48: 56–64, 1995.
18. **Jones CA, Petrovic N, Novak EK, Swank RT, Sigmund CD, and Gross KW.** Biosynthesis of renin in mouse kidney tumor As4.1 cells. *Eur J Biochem* 243: 181–190, 1997.
19. **Kagami S, Border WA, Miller DE, and Noble NA.** Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-β expression in rat glomerular mesangial cells. *J Clin Invest* 93: 2431–2437, 1994.
20. **Lai KN, Leung JCK, Lai KB, To WY, Yeung VT, and Lai FM.** Gene expression of the renin-angiotensin system in human kidney. *J Hypertens* 16: 91–102, 1998.
21. **Leehey DJ, Singh AK, Alavi N, and Singh R.** Role of angiotensin II in diabetic nephropathy. *Kidney Int* 77: 93–98, 2000.
22. **Leehey DJ, Song RH, Alavi N, and Singh AK.** Decreased degradative enzymes in mesangial cells cultured in high glucose media. *Diabetes* 44: 929–935, 1995.
23. **Makita Y, Ishidoh K, Kominami E, Funabiki K, Koide H, and Tomino Y.** Expression of cysteine proteinases, and their inhibitor, cystain β, in cultured rat mesangial cells. *J Diabetes Complications* 12: 328–336, 1998.
24. **Mendelsohn FA and Johnston CI.** A radiochemical renin assay. *Biochem J* 121: 241–244, 1971.
25. **Moe OW, Ujue R, Stan RA, Miller RT, Widell J, Alpern RJ, and Henrich Wl.** Renin expression in renal proximal tubule. *J Clin Invest* 91: 774–779, 1993.
26. **Muller MW, Todorov V, Kramer BK, and Kurtz A.** Angiotensin II inhibits renin gene transcription via the protein kinase C pathway. *Pflügers Arch* 444: 499–505, 2002.
27. **Neves FAR, Duncan KG, and Baxter JD.** Cathepsin B is a prorenin processing enzyme. *Hypertension* 27: 514–517, 1996.
28. **Nguyen G, Delarue F, Berrou J, Rondeau E, and Sraer JD.** Specific receptor binding of renin on human mesangial cells in culture increases plasminogen activator inhibitor-1 antigen. *Kidney Int* 50: 1897–1903, 1996.
29. **Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, and Sraer JD.** Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 109: 1417–1427, 2002.
30. **Park SH and Han HJ.** The mechanism of angiotensin II binding down-regulation by high glucose in primary renal proximal tubule cells. *Am J Physiol Renal Physiol* 282: F228–F237, 2002.
31. **Price DA, Porter LE, Gordon M, Fisher NDL, De'oliveira JMF, Laffel LMB, Passan DR, Williams GH, and Hollenberg NK.** The paradox of the low-renin state in diabetic nephropathy. *J Am Soc Nephrol* 10: 2382–2391, 1999.
32. **Re RN, MacPhee AA, and Fallon JT.** Specific nuclear binding of angiotensin II by rat liver and spleen nuclei. *Clin Sci (Lond)* 61, *Suppl* 7: 245S–247S, 1981.
33. **Reudelhuber TL.** Molecular biology of renin. In: *Molecular Nephrology*, edited by Schlondorff D and Bonventre JV. New York: Dekker, 1995, p. 71–89.
34. **Rohrwasser A, Morgan T, Dillon HF, Zhao L, Callaway CW, Hillas E, Zhang S, Cheng T, Inagami T, Ward K, Terreros DA, and Lalouel JM.** Elements of a paracrine tubular renin-angiotensin system along the entire nephron. *Hypertension* 34: 1265–1274, 1999.
35. **Schwobel J, Fischer T, Lanz B, and Mohaupt M.** Angotensin II receptor subtypes determine induced NO production in rat glomerular mesangial cells. *Am J Physiol Renal Physiol* 279: F1092–F1100, 2000.
36. **Sica DA and Bakris GL.** Type 2 diabetes: RENAAL and IDNT—the emergence of new treatment options. *J Clin Hypertens* 4: 52–57, 2002.
37. **Singh R, Alavi N, Singh AK, and Leehey DJ.** Role of antgiotensin II in glucose-induced inhibition of mesangial matrix degradation. *Diabetes* 48: 2066–2073, 1999.
38. **Singh R, Singh AK, Alavi N, and Leehey DJ.** Mechanism of increased angiotensin II levels in glomerular mesangial cells cultured in high glucose. *J Am Soc Nephrol* 14: 873–880, 2003.
39. **Song RH, Singh AK, and Leehey DJ.** Decreased glomerular proteinase activity in the streptozotocin in diabetic rat. *Am J Nephrol* 19: 441–446, 1999.
40. **Wolf G.** Molecular mechanisms of angiotensin II in the kidney: emerging role in the progression of renal disease: beyond haemodynamics. *Nephrol Dial Transplant* 13: 1131–1142, 1998.